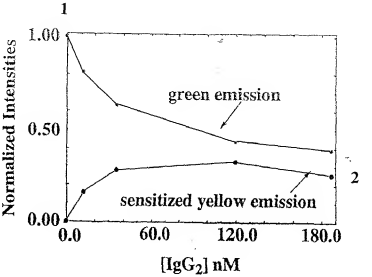
TOSOTT: EZ858660

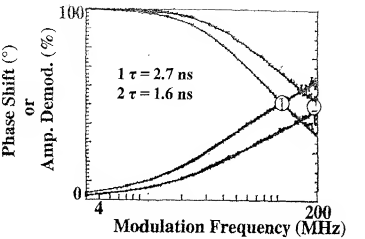
FIG. 1

FRET-Based Assays on Beads

Two color intensity measurement:
Titration of rhodamine tagged IgG to fluorescein tagged biomolecules



Phase sensitive detection of FRET:
Reduced lifetime of biomolecule fluorescence after binding to rhodamine-tagged IgG



EP 2 858 660 B1

FIG. 2

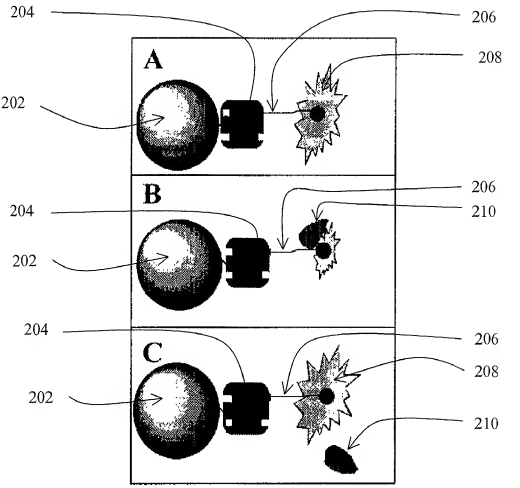


FIG. 3

Detection of Increasing Amounts of Red Tagged IgG in Affinity Column With Flow Cytometry Calibrated Beads

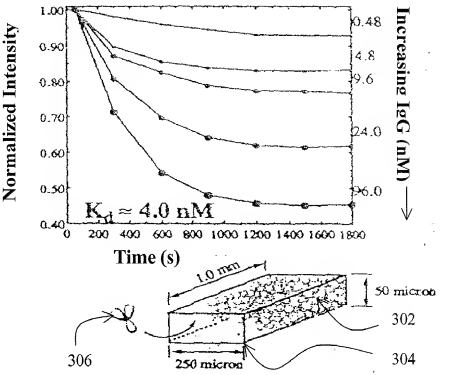


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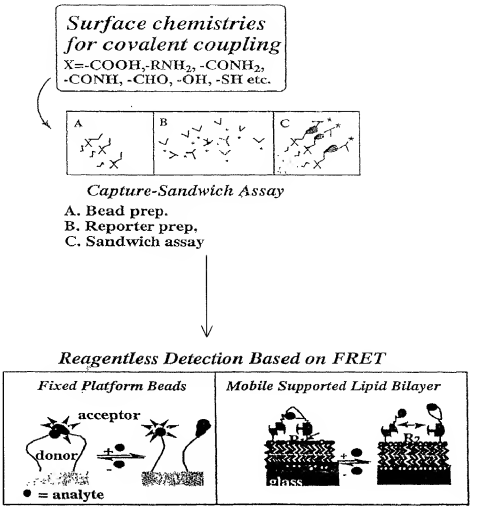
FIG. 4

Bead Based Flow Cytometric Assays



EP 2 090 672 A1

FIG. 5

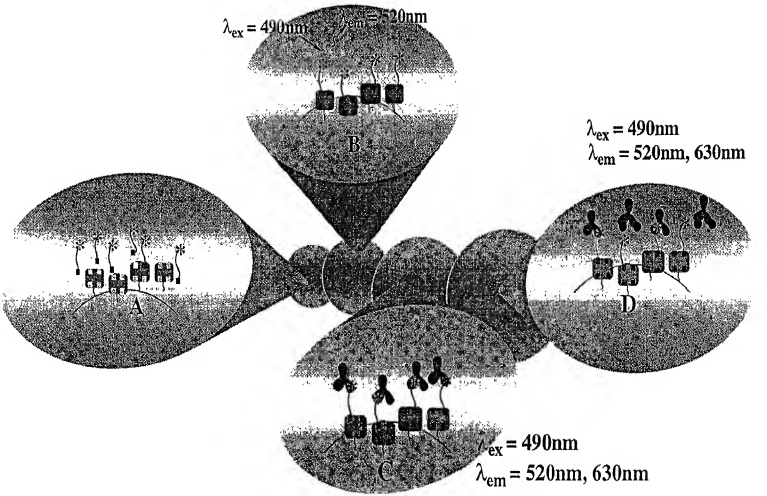


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FIG. 6

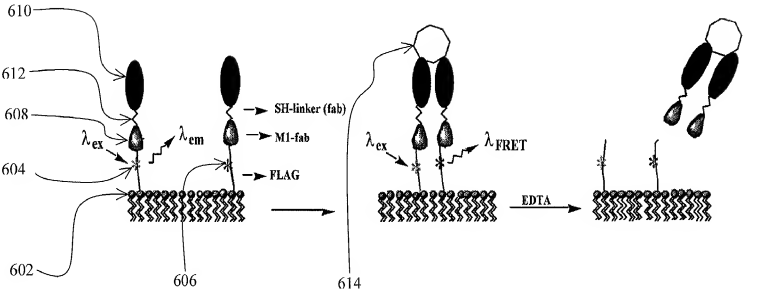
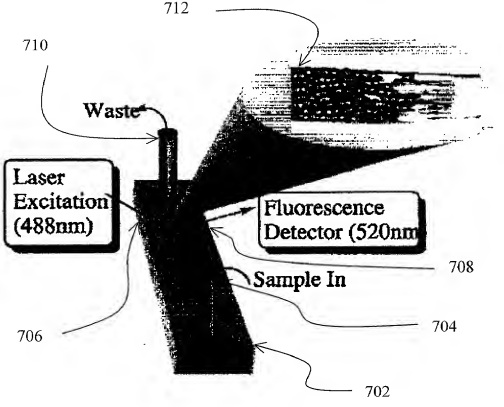


FIG. 7A



EP 0 971 217 A2

FIG. 7B

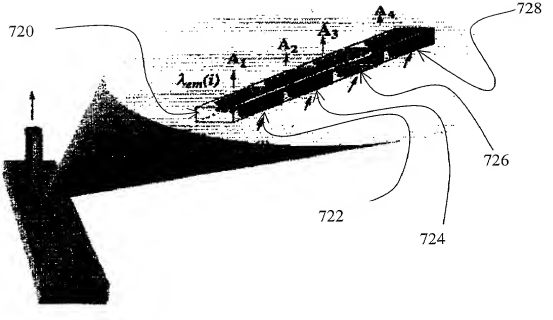


FIG. 8

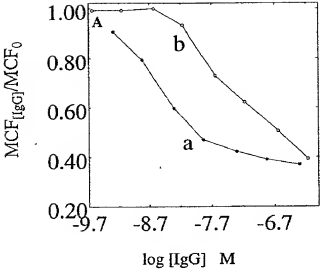

1.00
0.80
0.60
0.40
0.20
A
b
a
-9.7
-8.7
-7.7
-6.7
MCF[IgG]/MCF0
log [IgG] M

FIG. 9

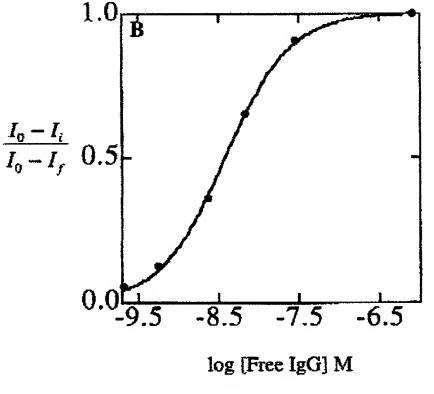

1.0
0.5
0.0
B
-9.5
-8.5
-7.5
-6.5
I0 − Ii / I0 − If
log [Free IgG] M

FIG. 10

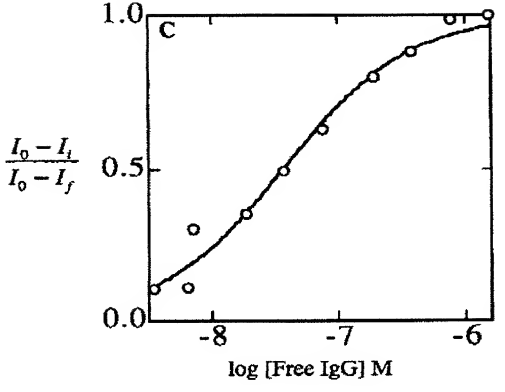


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FIG. 11

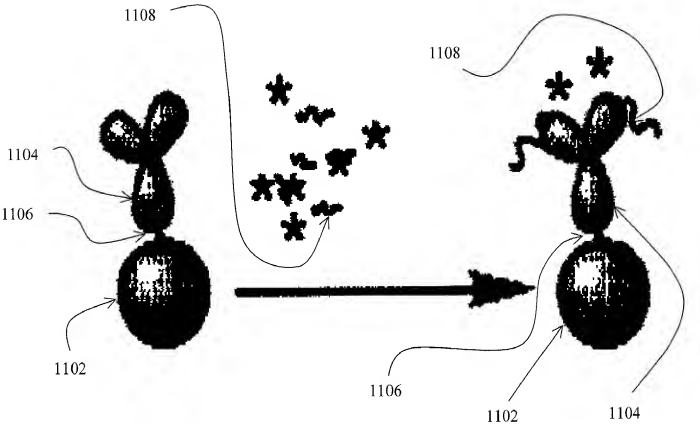

FIG. 12

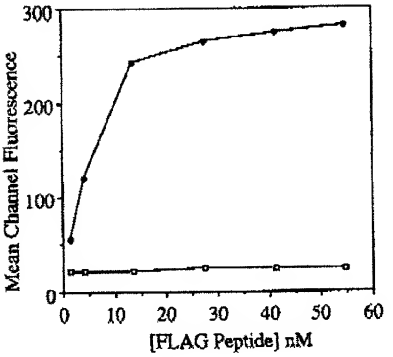
300
200
100
0
Mean Channel Fluorescence
0
10
20
30
40
50
60
[FLAG Peptide] nM

FIG. 13

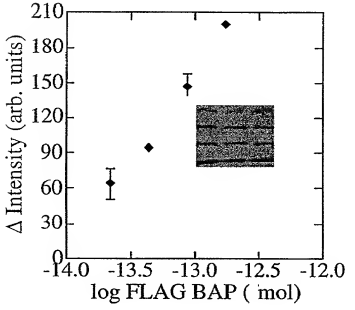
210
180
150
120
90
60
30
0
Δ Intensity (arb. units)
-14.0
-13.5
-13.0
-12.5
-12.0
log FLAG BAP (mol)

FIG. 14

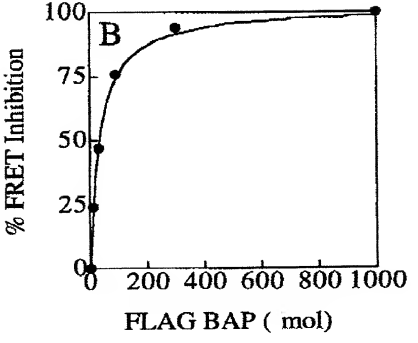

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FIG. 15

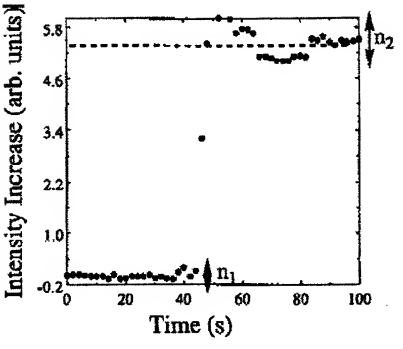
Intensity Increase (arb. units)
5.8
4.6
3.4
2.2
1.0
-0.2
0
20
40
60
80
100
Time (s)
n_2
n_1

FIG. 16

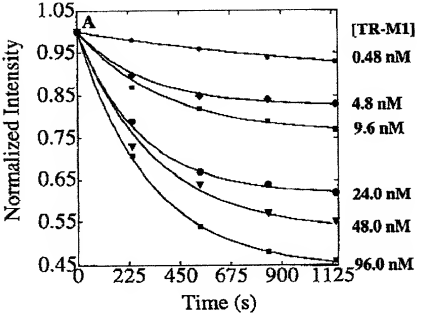
A
Normalized Intensity
1.05
0.95
0.85
0.75
0.65
0.55
0.45
0
225
450
675
900
1125
Time (s)
[TR-M1]
0.48 nM
4.8 nM
9.6 nM
24.0 nM
48.0 nM
96.0 nM

FIG. 17

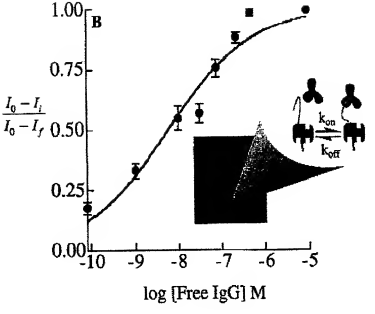

FIG. 18

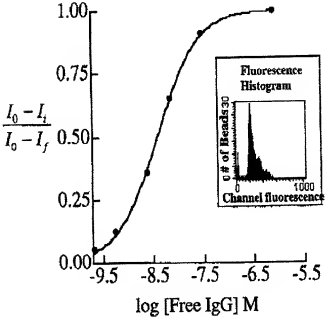

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FIG. 19

Characterization of Binding Affinities Between Beads, Flourescein Biotin, FLAG Peptides, and Antibodies

System	K_d(nM)
f biotin/beads	0.5
FLAG peptides/bead	
5-FLAG	0.30
6-FLAG	1.90
TR-M1/5-FLAG (solution)	9.0
TR-M1/5-FLAG (beads + 1mM Ca^{2+})	4.0
TR-M1/5-FLAG (beads no Ca^{2+})	37.0
M2/5-FLAG (beads)	8.0